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Cornell University
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373 Pine Tree Road
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UU	UU	UU	UU		19b. TELEPHONE NUMBER 607-254-8533

RPPR Final Report
as of 25-Feb-2019

Agency Code:

Proposal Number: 66998LS **Agreement Number: W911NF-15-1-0268**
INVESTIGATOR(S):

Name: Peng Chen
Email: pc252@cornell.edu
Phone Number: 6072548533
Principal: Y

Organization: **Cornell University**
Address: Office of Sponsored Programs, Ithaca, NY 148502820
Country: USA
DUNS Number: 872612445 EIN: 150532082

Report Date: 30-Sep-2018 Date Received: 07-Sep-2018
Final Report for Period Beginning 01-Jul-2015 and Ending 30-Jun-2018
Title: Imaging Efflux Machineries for Metal Defense in Live Bacteria
Begin Performance Period: 01-Jul-2015 **End Performance Period:** 30-Jun-2018
Report Term: 0-Other

Submitted By: Peng Chen Email: pc252@cornell.edu
Phone: (607) 254-8533

Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 2 **STEM Participants:** 2

Major Goals: Please see uploaded pdf

Accomplishments: Please see uploaded pdf.

Training Opportunities: This project has provided training for graduate students (Lauren Genova. Feng Yang) and postdocs (Ace George Santiago, Joshi Chandra, Kushal Sengupta, Xianwen Mao, and Bing Fu).

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Results Dissemination: Publications:

1) A. G. Santiago, T.-Y. Chen, L. Genova, W. Jung, A. Thompson, M. McEvoy, P. Chen* “Adaptor protein mediates dynamic pump assembly for bacterial metal efflux” Proc. Natl. Acad. Sci. U.S.A. 2017, 114, 6694-6699.
2) T.-Y. Chen,* Y.-S. Cheng, P.-S. Huang, P. Chen* "Facilitated unbinding via multivalency-enabled ternary complexes: New paradigm for protein-DNA interactions" Acc. Chem. Res. 2018, 51, 860-868.
3) F. Yang, T.-Y. Chen, L. Krzeminski, A. G. Santiago, W. Jung, P. Chen* “Single-molecule dynamics of the molecular chaperone trigger factor in living cells” Mol. Microbiol. 2016, 102, 992-1003.
4) T.-Y. Chen, W. Jung, A. G. Santiago, F. Yang, ?. Krzemi?ski, P. Chen* “Quantifying multi-state cytoplasmic molecular diffusion in bacterial cells via inverse transform of confined displacement distribution” J. Phys. Chem. B 2015, 119, 14451-14459.
5) D. J. Martell, C. P. Joshi, A. Gaballa, A. G. Santiago, T.-Y. Chen, W. Jung, J. D. Helmann, P. Chen* “Metalloregulator CueR biases RNA polymerase’s kinetic sampling of dead-end or open complex to repress or activate transcription” Proc. Natl. Acad. Sci. U.S.A. 2015, 112, 13467-13472.

Media coverage:

"E. coli bacteria's defense secret revealed" Cornell Chronicle, June 12, 2017.

Seminar presentations.
1. Frontiers in biophysics, Cornell University, September 12, 2015
2. Department of Chemistry, University of Pittsburgh, November 5, 2015
3. Department of Chemistry, University of California at Berkeley, January 12, 2015
4. Natural Sciences Faculty Colloquium, Tata Institute of Fundamental Research, Mumbai, India, February 15, 2016
5. Indian Association for the Cultivation of Science, Kolkata, India, February 16, 2016
6. Army Research Laboratory, March 1, 2016
7. Excellence in Catalysis Award Lecture, Catalysis Society of Metro NY, May 18, 2016
8. Department of Chemistry, Penn State University, September 8, 2016.
9. Department of Chemistry, University of Utah, October 3, 2016
10. Departamento de Química, Cinvestav, Mexico City, October 24, 2016
11. Department of Chemical and Environmental Engineering, Yale University, November 9, 2016
12. Department of Chemistry, University of Wisconsin at Madison, January 31, 2017
13. Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, July 5, 2017
14. Department of Chemistry, Nanjing University, China, July 6, 2017
15. Catalysis forum, State Key Laboratory of Catalysis, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, July 10, 2017
16. Colloquium of the Visiting Professor in the Debye Chair, Utrecht University, September 21, 2017
17. Department of Physics, Chalmers University of Technology, Sweden, November 20, 2017
18. Nanoscale Photonic Imaging Collaborative Research Center, Georg August University, Göttingen, Germany, November 28, 2017
19. Institute for Molecules and Materials, Radboud University, the Netherlands, November 29, 2017
20. Department of Chemistry, University of Texas at Austin, February 21-22, 2018
21. Division of Chemistry and Chemical Engineering, CalTech, February 26-27, 2018
22. Center for Chemistry at the Space-Time Limit, UC Irvine, March 15, 2018
23. Department of Chemistry and Biochemistry, UC San Diego, March 16, 2018
24. Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, April 13, 2018
25. Department of Chemistry, Seoul National University, South Korea, June 5, 2018
26. Department of Chemistry, Gwangju Institute of Science and Technology, South Korea, June 7, 2018

Invited, relevant conference talks
1. Gordon Research Conference: Cell Biology of Metals, Mount Snow Resort in West Dover, VT; July 26-31, 2015
2. Keynote speaker, Lorentz workshop on “Proteins and Beyond,” Leiden, the Netherlands; October 12-16, 2015
3. Symposium on “Metal ions and protein function: theoretical models and applications,” PacifiChem 2015; Honolulu, HI; December 15-20, 2015
4. 6th International Conference on Metals in Genetics, Chemical Biology and Therapeutics (ICMG 2016), Indian Institute of Science, Bangalore, India; February 17-20, 2016.
5. Symposium on “Understanding Enzymatic Catalysis across Multiple Timescales: Experiment and Theory,”

ACS National Meeting, San Diego, CA; March 13-17, 2016
6. Symposium in honor of Edward Solomon on winning Alfred Bader Award in Bioinorganic or Bioorganic Chemistry, ACS National Meeting, San Diego, CA; March 13-17, 2016
7. Copper 2016 Sorrento, 10th International Copper Research Meeting, Sorrento, Italy; September 25-30, 2016
8. V Latin American Meeting on Biological Inorganic Chemistry (V LABIC), Querétaro, Mexico; October 18-22, 2016
9. The Pittsburgh Spectroscopy Award Symposium, Pittcon, Chicago, IL; March 5-9, 2017
10. ARO-NASA Life Sciences Basic Research Review, Mountain View, CA; July 31 - August 3, 2017
11. Symposium on "Electronic structure contributions to function: from metals in biology to materials science," Division of Inorganic Chemistry, ACS National Meeting, Washington, D.C.; August 20-24, 2017
12. Plenary Speaker, Symposium of the Center for Chemical Dynamics in Living Cells, Chung-Ang University, Seoul, South Korea; June 4, 2018
13. Keynote lecturer, 14th European Biological Inorganic Chemistry Conference (EuroBIC-14), University of Birmingham, UK; August 26-30, 2018
14. Biochemical Society Harden Conference on Single Molecule Bacteriology (SMOLBAC), Oxford, UK; September 9-12, 2018
15. Copper 2018, 11th International Copper Meeting, Sorrento, Naples, Italy; September 23-28, 2018

Honors and Awards: Bau Family Award in Inorganic Chemistry (2018)
Sessler Distinguished Alumni Lecturer, Stanford University (2018)
Visiting Professor in the Debye Chair, Utrecht University (2017)
Catalysis Forum Lecturer, DICP, Chinese Academy of Sciences (2017)
Excellence in Catalysis Award, Catalysis Society of Metro New York (2016)
NSF Colloquium Lecturer, Tata Institute of Fundamental Research (2016)

Plenary lecturer, 16th Conference on Methods and Applications of Fluorescence (2019)
Keynote Lecturer, International Bunsen Discussion Meeting on Probing Chemical Reactions by Single-molecule Spectroscopy (2019)
Keynote Lecturer, 14th European Biological Inorganic Chemistry Conference (2018)
Plenary Speaker, IBS Symposium on Nanomaterials and Spectroscopy, Korean Advanced Institute of Science and Technology, South Korea (2018)
Plenary Speaker, Symposium of the Center for Chemical Dynamics in Living Cells, Chung-Ang University, South Korea (2018)
Plenary Speaker, 6th International Congress on Operando Spectroscopy (2018)

Editorial advisory board, ACS Chem. Biol. (1/2016-12/2018)
Editorial advisory board, Chem. Phys. Lett. (2014-2020)
Regular Member, NIH Enabling Bioanalytical and Imaging Technologies (EBIT) Study Section (07/01/17 – 06/30/23)

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: Graduate Student (research assistant)
Participant: Lauren Genova
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Feng Yang

Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Chandra Joshi
Person Months Worked: 7.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Xianwen Mao
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Kushal Sengupat
Person Months Worked: 10.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Bing Fu
Person Months Worked: 5.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Ace George Santiago
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: PD/PI

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Participant: Peng Chen
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Major Goals

Our *objective* is to define the connection between the assembly forms of CusCBA and the cellular demands for Cu efflux in live *E. coli* cells, as well as the dynamic interconversions among the different assembly forms under various Cu efflux demands, with the *long-term goal* of understanding how bacterial membrane efflux pumps can be manipulated for antibacterial treatments.

Accomplished under Goals

We have implemented nanometer-resolution single-molecule tracking of CusA tagged with the photoconvertible fluorescent protein mEos3.2 to quantify its diffusion behaviors associated with different assembly forms in living *E. coli* cells. We have completed our objective and published our major results in *PNAS* (**2017**, *114*, 6694-6699), which has also been highlighted by Cornell Chronicle news article "*E. coli* bacteria's defense secret revealed" *Cornell Chronicle*, June 12, 2017.

In summary, we have found:

1) In the absence of metal stress (when efflux is not needed), the inner-membrane pump CusA has a significant population in the disassembled state in the cell, allowing for periplasmic plasticity. Under metal stress, the population of CusA dynamically shifts toward the assembled state, allowing for the formation of intact CusCBA complexes for metal efflux.
2) The periplasmic adaptor protein CusB, the metal-sensing protein in the CusCBA system, is responsible for mediating complex assembly. This adaptor protein-mediated dynamic pump assembly allows the bacterial cell for efficient efflux on cellular demand while still maintaining periplasmic plasticity.
3) While CusCBA is a member of the tripartite resistance-nodulation-division (RND) family of efflux pumps, we believe these findings are broadly relevant to other multicomponent efflux systems and may provide opportunities for antibacterial treatments that inhibit efflux complex assembly.

Novelty: The following aspects make our study novel:

1) Our study represents the *first example*, to our knowledge, of (a) that a multicomponent membrane efflux pump exists in a dynamic assembly equilibrium *on its own* in a living cell with or without stress by the substrate, and (b) that the periplasmic adaptor protein is the key substrate-responsive element. This mechanism gives insight into how cells maintain efflux functions and membrane plasticity simultaneously.
2) Our study is the *first report* that the efflux pumps respond to external toxic molecules earlier than the gene activation of the associated operon. Therefore, this adaptor-protein-mediated assembly serves as the first responder when the cell needs to immediately expel toxins.
3) Our study combines single-molecule super-resolution imaging with genetic engineering, which circumvents the general challenge in studying membrane proteins, whose *in vitro* reconstitution is technically demanding, especially for tripartite complexes that involve two membrane proteins and a periplasmic protein.

Broad relevance and impact: Multicomponent efflux pumps are ubiquitous in Gram-negative bacteria and confer bacteria multi-drug resistance (e.g., the ATP-binding cassette superfamily and the major facilitator superfamily, aside from the RND superfamily). The substrate-binding capabilities of some adaptor proteins in these families could possibly allow them to mediate the substrate-responsive dynamic assembly of these efflux complexes, making our mechanism likely general. This mechanism may also provide opportunities for antibacterial treatments that inhibit efflux complex assembly.